Capillary HPLC separation of 2D in-gel digested protein spots from proteome projects

Biopharmaceutical

Proteome research is rapidly gaining importance for target identification in drug discovery and drug development, process optimization for recombinant proteins and for diagnostic purposes (for example, tumor markers). The main reasons are that the level of gene expression does not represent the amount of active protein in a cell, that the gene sequence does not describe posttranslational modifications which are essential for the function and activity of a protein and that the genome of an organism does not describe dynamic processes on the protein level.

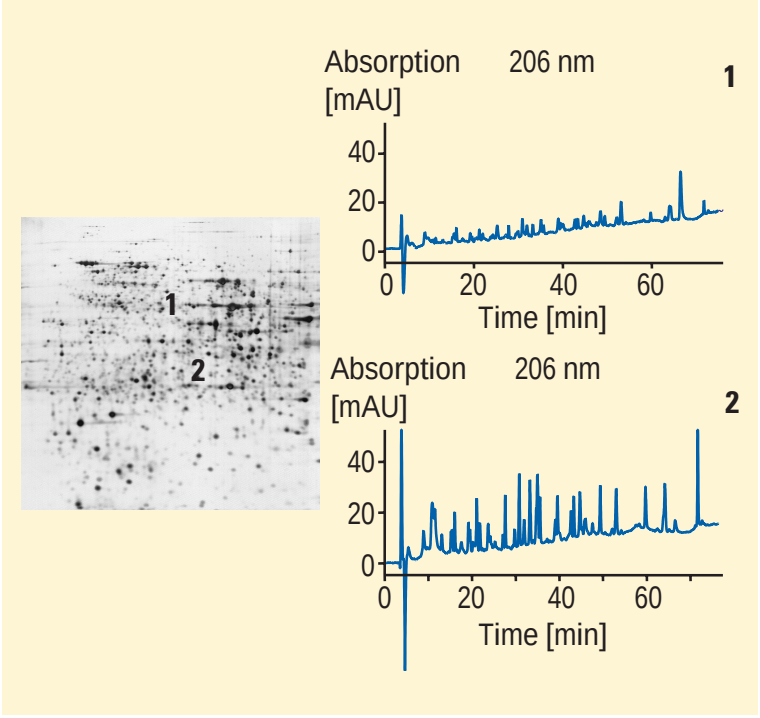


Figure 1
Characterization of protein spots from a 2D gel. About 2 mg total protein were separated, resulting in nearly 800 coomassie stainable spots (left side). After in-gel digestion the resulting peptide maps of two randomly selection protein spots are separated on 180-µm id capillary columns.

Conditions

Column
0.18 x 250 mm Vydac C-18 (LC Packings, The Netherlands)
Mobile phase
A = 0.025 % TFA
B = acetonitrile, 0.020 % TFA
Flow rate
0.1 ml/min with flow split to 2 µl/min
Flow splitter
VAR-100 (LC Packings, The Netherlands)
Gradient
0–75 min 2–4 % B
UV detector
diode array detector
206 nm, 280 nm
spectra 200–320 nm
500 nl flow cell kit
Temperature
ambient

In proteomics, complex protein mixtures such as complete cells or only partially prefractionated mixtures are separated first by 2D gel electrophoresis. After separating the proteins by their characteristic pI-values and molecular weights the proteins are stained typically with silver for analytical purposes (qualitative and quantitative evaluation of the protein pattern) or with colloidal coomassie for further microanalytical characterization of protein spots of interest. Typically, proteins are cleaved in the gel matrix with protease trypsin. After digestion the peptide mixture is eluted out of the gel and analyzed. If the genome of an organism is known, mainly peptide mass fingerprint analysis using MALDI mass spectrometry is applied. However, if the genome is not or only partially known, peptides have to be separated and characterized by Edman sequencing.

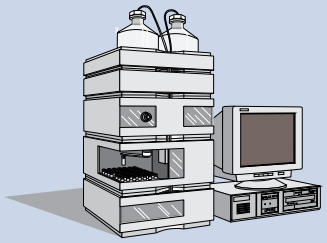
Most of the protein spots in a 2D gel are present in amounts only little above one picomol, but mainly at the femtomol level. To separate femtomol peptide maps capillary HPLC is the only method of choice. Typically, 300-µm id, or at least 180-µm id capillary columns for higher sensitivity, have to be used. The Agilent 1100 Series system using the LC Packings flow splitting device can easily produce reproducible flow rates of 4 µl (for 300-µm id columns) or 2 µl (for 180-µm id columns) per minute starting with flow rates of only 100 µl per minute before split.

The reversed-phase separations of two in-gel digested 2D protein spots on a 180-µm id capillary column is shown in figure 1. Separation of peptide map 1 corresponds to only about 250 fmol, and separation of peptide map 2 corresponds to about 750 fmol peptide material. Individual peptides could be collected and analyzed further analyzed with high sensitivity protein sequencer systems.

Equipment

Agilent 1100 Series

- Binary pump (includes vacuum degasser)
- Autosampler
- Diode array detector capillary flow cell 10-mm path length, 500 nl cell volume
- Agilent ChemStation + 3D software



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